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Striate palmoplantar keratoderma resulting from a missense mutation in *DSG1*

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Running title: SPPK resulting from missense mutations in DSG1

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Dear Editor, palmoplantar keratodermas (PPKs, OMIM #144200) refer to a large phenotypically and genetically heterogeneous group of keratinisation disorders characterised by marked hyperkeratosis on the surface of the palms and soles.[1] Striate palmoplantar keratoderma (SPPK) features linear hyperkeratosis of the volar aspects of the fingers, extending onto the palms, as well as focal plantar keratoderma. The disease is most commonly caused by heterozygous mutations in *DSG1* encoding desmoglein 1 (DSG1)[2] (SPPK1, OMIM #148700) but can also arise from mutations in *DSP* encoding desmoplakin (DSP)[3] (SPPK2, OMIM #612908) or *KRT1* encoding keratin 1 (K1)[4] (SPPK3, OMIM #607654). To date, more than 25 mutations in *DSG1* have been identified in patients with SPPK1. These mutations were uniformly found to result in a premature termination codon (PTC).[5,6]
In this study, we present, to our knowledge, the first case of SPPK caused by a missense (rather than PTC-causing) mutation affecting a residue predicted to be of critical importance for DSG1-mediated adhesion.

The patient was a 27-year-old female of Jewish origin. She reported thickening of the skin of palms and soles since 1 year of age, resulting in pain while walking. She denied any additional systemic or dermatologic manifestations apart from yellow discolouration of all toenails. Her father and her sister reportedly displayed similar clinical features. On examination, the patient exhibited foci of plantar yellowish fissured hyperkeratotic skin involving weight bearing areas as well as subtle linear

hyperkeratosis of the volar aspects of the fingers (Fig. 1a). Haematoxylin and eosin staining of a skin biopsy showed hypergranulosis, marked orthohyperkeratosis and widening of the intercellular spaces between keratinocytes. (Fig. 1b).

To identify the causative mutation underlying SPPK in the patient, after obtaining ethical approval and informed consent, we used direct sequencing of gDNA to fully sequence all coding exons of *DSG1, KRT1, DSP and KRT16.* We discovered a heterozygous A to G transition at position 254 (c.254A>G) within exon 4 (Fig. 1c) of the *DSG1* gene *(*reference sequence Ensembl accession number ENST00000257192.4), resulting in a p.Y85C substitution. The sequence variation was not detected in 629 Jewish healthy controls and was not found in the following genomic databases: NCBI, UCSC, HGMD, ESP, ExAC, 1000 genomes and ENSEMBL, comprising a total of more than 58,000 individual sequences. The p.Y85C variation is predicted to be deleterious by two prediction tools (Polyphen2 score 1, range 0–1; SIFT scores 0, range 1–0) and was found to affect a highly conserved residue (Conseq score = 9, range 1-9). No mutations were identified in *KRT1*, *DSP* or *KRT16*.

To assess the consequences of the mutation, we modeled the wildtype and the mutant DSG1 proteins using three prediction tools (Fig. 1d): SWISS PROT[7] and LOMETS[8] predictions were based on the crystal structure previously determined for the human DSG2 ectodomain (PDB code 5ERD, chain A, 36% identity) while PHYRE2[9] was used based on the human DSG3 ectodomain (PDB code 5EQX, chain A, 55% identity). Similarity between the model structures was assessed using PyMOL alignment (The PyMOL Molecular Graphics System, Version 1.8

Schrödinger, LLC).

Like other members of the cadherin superfamily, desmosomal cadherins are thought to form trans-adhesive dimers, which rely on the interaction between the conserved tryptophan (W2) of one molecule and a specific hydrophobic pocket, serving as an acceptor site, on the interacting molecule from the opposing cell.[10-13] This was recently shown to depend predominantly on a heterotypic interaction between the donor site of a desmocollin molecule and the acceptor pocket of a DSG molecule,[14] which is lined by the side chains of specific conserved residues including Y85 (numbered Y36 in the final DSG chain).[12-14]

Accordingly, we found that the affected amino acid, Y85, resides within the first extracellular domain (EC1) of the protein (Fig. 1e), inside a hydrophobic-positively charged area (Fig. 1f). The p.Y85C mutation could affect the binding capability of the hydrophobic pocket in several ways. First, while the hydroxyl side chain of Y85 was found to reach the inner part of this pocket, the C85 mutation is lacking the length to do so (Fig. 1f). Additionally, the mutant C85 residue is predicted to aberrantly interact with C127, a part of the adhesive interface of the pocket,[13] and could potentially form a disulfide bond either through an intramolecular cis-interaction, due to proximity of the two amino acids, or through a trans-interaction with another bound desmosomal cadherin. When we used the same modeling tools to examine the effect of p.V83D and p.R86C, both highly rare heterozygous non-pathogenic variants in close proximity to p.Y85C, we discovered that although residing close to the hydrophobic pocket, they are not predicted to disturb its binding capability or to have an effect on DSG1 dimerisation (Fig. 1g).

To conclude, we describe here the first case of SPPK resulting from a heterozygous missense (as opposed to nonsense or frameshift) mutation in the *DSG1* gene to our knowledge. Protein modeling predicts this mutation to abolish DSG1-mediated adhesive function, thus resulting in what could be manifested as functional haploinsufficiency. Of interest, DSG1 has been shown to downregulate the Ras/MAPK pathway by interacting with Erbin; elevated Ras activity, resulting from DSG1 deficiency, is therefore thought to trigger cellular mechanisms leading to hyperkeratosis in SPPK.[15] Whether missense mutations in the extracellular domain affect this regulatory pathway remains to be determined. In addition to the relevance of these data to PPK diagnostics, the putative mode of action of the mutation identified in the present study emphasises the importance of the role of DSG1-mediated adhesive function in palmoplantar skin.

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Legends to figures

Figure 1. Clinical features, mutation analysis and protein modeling. (a) The patient presented with foci of plantar hyperkeratosis involving weight bearing areas with multiple skin fissures (left panel). Linear hyperkeratosis of the volar aspects of the fingers was also observed (right panel); (b) Hematoxylin and eosin staining of a skin biopsy obtained from the skin of the patient demonstrates hypergranulosis, marked orthohyperkeratosis and widening (arrows) of the intercellular spaces between keratinocytes, compatible with keratoderma (scale bar = 100 μm); (c) Direct sequencing of gDNA revealed a A>G transition at position c.254 within exon 4 of *DSG1* (upper panel), compared to wild-type sequence (lower panel). (d) Comparison between the molecular model of the human DSG1 protein based on three prediction tools: LOMETS (green), SWISS PROT (magenta) and PHYRE2 (cyan) shows that all the structures are similar (RMSD<4 with PyMOL alignment) and that the Y85 amino acid is located in the first domain (in red, highlighted by black oval). (e) A schematic representation of the DSG1 molecule shows that the p.Y85C mutation is located

inside the EC1 domain of the protein. EC, extracellular domain; EA, extracellular anchoring domain; P, propeptide region; SP, signal peptide; TM, transmembrane domain (protein scheme modified from Hershkovitz et al[5]); (f) Mutation modeling was performed based on the above models. The protein molecules are overlaid with semi-transparent calculated electrostatic potential (blue-positive, red-negative) created using PyMOL. Y85 (in red) is located in a hydrophobic-positively charged pocket-shaped surface, predicted to serve as a pathway for DSG1 binding. While the hydroxyl of the tyrosine lies within the inner part of the pocket (left panel), the mutant C85 substitution is lacking the length to reach its inner rim (right panel). (g) Modeling of two rare heterozygous variants in close proximity to Y85 (in red) was performed based on the above models. The p.V83D variant (in purple, right panel) resides further away from the hydrophobic pocket and will lead to a negatively charged glutamic acid that is not predicted to alter the negatively charged area of the wild type p.V83 (in purple, left panel). p.R86 (in green, left panel) is located in proximity of the hydrophobic pocket and of p.C127 (in black) but faces the opposite direction. The p.R86C variant (in green, right panel) is not predicted to affect the binding capability of the hydrophobic pocket or to form a disulphide bond with p.C127.